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Disorders of reproduction in epilepsy—What can we learn from animal studies?

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Summary Several animal studies have shown that both the epilepsy itself and many antiepileptic drugs (AEDs) affect reproductive endocrine function in both males and females. Epileptic activity may lead to arrested ovarian cyclicity, anovulatory cycles, polycystic ovaries, and endocrine changes in female animals. In males, seizures disturb normal reproductive physiology by inducing endocrine changes, alterations in gonadal size, and hyposexuality. Several AEDs also affect endocrine function, fertility, and gonadal morphology in both sexes. This paper reviews the literature regarding animal studies related to reproductive disorders in epilepsy. Although care should always be taken when applying data from animal experiments to the human situation, animal models provide a unique possibility for investigating the independent effects of the epilepsy itself and the effects of AEDs in isolation, without confounding factors. By constantly comparing results from clinical and animal studies, and by developing appropriate animal models, several mechanistic questions regarding the complex interplay between epilepsy, hormones, and AEDs can be explored. Animal experiments should play an integral part in the study of reproductive endocrine disorders in epilepsy.

Introduction

Reproductive endocrine dysfunction is unusually common among women and men with epilepsy.[1] In women, menstrual disorders, polycystic ovaries, and infertility have been described,[1—3] while in men, reduced potency and sperm abnormalities have been found.[1,4,5] In both sexes, sexual problems[6] and endocrine changes are frequently described.[1,2,3]

The reasons for the changes in reproductive endocrine function are multifactorial, including both the epilepsy itself and the antiepileptic medication. In addition, confounding factors like loss of self-confidence, social rejection, and other psychosocial problems may affect endocrine function and reproductive health.

Animal studies allow us to investigate the independent effects of the epilepsy itself and the effects

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of antiepileptic drugs (AEDs) in isolation, without confounding factors. The effects of the individual drugs can be studied at different doses and time intervals, and possible cellular and genetic mechanisms of action of the different drugs can be studied in detail.

Reproductive endocrine effects of epilepsy

It is well known that epilepsy itself affects the secretion of pituitary hormones.[1,7] Even the laterality of epileptic activity may be of importance as left-sided temporal foci seem to increase the occurrence of polycystic ovaries in women, while right-sided foci increase the frequency of hypogonadotropic hypogonadism.[8]

In a series of experiments, Edwards et al.[9,10] showed how seizures affected reproductive function in both female and male rats. In females, amygdala-kindled seizures arrested ovarian cyclicity in all animals. The animals also had high serum estradiol, increased pituitary weight, and polyfollicular ovaries consisting of many cystic follicles, as well as follicles in various stages of growth and atresia. Progesterone treatment of kindled females resulted in a different endocrine profile than that in non-progesterone-treated, kindled rats with higher serum estradiol, testosterone, and prolactin levels. The animals had an increase in pituitary weight, polycystic ovaries, and thecal cell hyperplasia. Further, bilateral ablations of the basolateral portion of the amygdala in adult female deer mice can induce anovulatory cycles and polycystic ovaries.[11] Stimulation of the corticomedial amygdala can induce ovulation and uterine contractions in several species,[12,13] while bilateral amygdalectomy in female monkeys induces amenorrhea and hypogonadal vaginal changes.[14]

Electroconvulsive seizures have been shown to delay the onset of puberty, to attenuate the responsiveness of luteinising hormone (LH) and follicle-stimulating hormone (FSH) to gonadal feedback, and to increase prolactin release in female rats.[15]

In male rats, amygdala-kindled seizures resulted in an increase in serum testosterone, estradiol, and prolactin in intact males, accompanied by a significant increase in testis, epididymis, and pituitary weight, as well as a significant decrease in prostate weight.[9] Maximal electroshock seizures (MES) treatment caused a short-term reduction in serum testosterone and a decrease in testis, epididymis, and prostate weight. These results indicate that both focal limbic (amygdaloid) seizures and generalized MES seizures disturb normal reproductive physiology in the male rat. In cats, it has been shown that epileptic activity in the temporal lobe induces hyposexuality.[16] Bilateral amygdalectomy in both adult male rats and cats results in marked degeneration of the testes.[17]

Reproductive endocrine effects of AEDs

Animal studies on the effect of AEDs on reproduction can be divided into studies on fertility, semen quality, gonadal morphology, and endocrinology. Due to the wide variation in study design, dosage regimens, and length of treatment, some studies may show apparently conflicting results. Here we outline some of the main trends in animal research on this topic, with particular focus on those long-term studies which would be of greatest relevance and interest to clinical practice.

Fertility

It has been claimed that fertility rates have been reduced after long-term treatment with different AEDs. In one study, fertility rates in rats after 60 days low-dose drug treatment with either carbamazepine (CBZ) or valproate (VPA) was reduced from 90% in controls to 40% and 30%, respectively.[18] An effect of VPA on fertility rates was also observed by Cohn et al.,[19] in a study in which the fertility rates of young male rats was investigated after 3-months of treatment with phenytoin (PHT), clonazepam (CZP), VPA, or CBZ. A significantly reduced fertility rate was only seen for VPA. The VPA dose used was 30 mg/(kg day), compared with 20 mg/kg for PHT and CBZ, and 1.8 mg/kg for CZP, but apparently serum concentrations were not measured. In contrast, another study reported that treating male rats with VPA doses of up to 500 mg/kg for 10 weeks had no effect on the number of pregnancies achieved.[20] However, it should be noted that fertility rate itself is a weak indicator of toxicity on male reproductive organs.[21] Direct measures of female animal fertility after AED treatment have not been studied in detail.

Fertility is dependent on sexual activity, a regular oestrus cycle or mating season, and on semen quality. Very few studies have addressed the issue of sexual activity in animals after AED treatment, but doses of VPA of up to 500 mg/(kg day) for 10 weeks did not seem to reduce copulation in rats.[20] Soliman and Abla Abd,[18] however, reported that sexual desire was reduced in rats treated with either CBZ or VPA at very low doses.

Little information is available about possible associations between AEDs and oestrous cycle irre-

gularities. The oestrous cycle was not affected in female Wistar rats treated with daily doses CBZ of 5, 10, or 100 mg/kg for 21 days,[22] nor has any effect of VPA on cycle regularity been observed in rats or monkeys.[23,24] However, for both these latter studies, it is uncertain whether the drug concentrations were within the therapeutic range for a sufficient period of time to induce clinically relevant effects. Due to the much higher metabolic rate for the drug in these animals, it is probable that the VPA concentrations were well below the therapeutic range for most of the day, and this is one of the major problems of all animal studies investigating endocrine effects of AEDs.

Semen quality

A wide range of so-called "old" AEDs have been claimed to reduce semen quality in a series of different animal studies.[18,19,20,25,26] The lack of negative effects on semen quality presently reported for "new" drugs does not imply that they have been proven to be *without* such effects, but merely reflects the lack of relevant data obtained to date. In animal studies on the effects of AEDs on semen quality, which have mostly been performed in rats, the study duration is very important. It has been shown that a 4-week drug treatment period is necessary to examine effects of AEDs on fertility in rats,[27] and in order to include a complete spermatogenetic cycle in the rat, which is 50–60 days, a 60-day treatment period should be used as a minimum.[28]

Two studies on rats have shown a reduction in total sperm count, varying from 20% to 40%, after long-term treatment with CBZ, possibly related to different dosing regimens.[18,19] CZP treatment of rats also significantly reduced total sperm count.[19] It is, however, important to note that in the same study neither CZP nor CBZ reduced the fertility rate of the rats.

Considerable interest has been directed towards the possible effects of VPA on semen quality, which has been found to be reduced after long-term treatment in mice and rats.[18,19,20,25] Both the total number of sperm and the number of motile sperm have been found to be significantly reduced in several different animal studies.[18,19,20,25] Additionally, an increase in the number of morphologically abnormal spermatozoa has been recorded, with an increase in sperm tail abnormalities.[18]

To study the effects of VPA in another species, we recently conducted a study using male goats as the animal model.[26] Nine bucks, aged 2 months at the start of the study, received VPA mixture twice daily for 8 months. Serum concentrations of VPA 2 h after last dose were about 700 μmol/l, after 4 h about 500 μmol/l, and after 6 h about 300 μmol/l. A significantly higher proportion of sperm from VPA-treated bucks showed abnormal chromatin, as compared with controls, demonstrating an adverse effect of VPA treatment on DNA. Both semen volume, total sperm count, and sperm motility tended to be lower in the VPA-treatment group, as compared with controls, but the differences were not significant, which was probably because of the relatively low number of animals included in the study.

Effects of AEDs on gonadal morphology

Testis and male accessory sex glands

In one of the very few studies comparing the effects of different AEDs on gonadal morphology, male rats were fed with PHT (20 mg/kg), CZP (1.8 mg/kg), VPA (30 mg/kg), or CBZ (20 mg/kg) daily for 3 months.[19] No changes in testicular weight were observed in any of the study groups. There was, however, a significant decrease in prostate weight in the rat groups treated with VPA or CBZ, while VPA treatment was also associated with a reduction in epididymis weight. The latter finding was further reflected in reduced sperm content and motility.[19]

Most interest regarding the effect of AEDs on testicular morphology has revolved round VPA. Chronic toxicity studies from the 1970s, performed by Abbott laboratories, apparently indicated testicular damage, including degeneration of the interstitial cells after VPA treatment.[29] In an early study by Walker et al.,[30] 13-week VPA treatment of rats, at 1200 and 1600 mg/(kg day), resulted in testicular atrophy. In dogs treated for 13 weeks, testicular atrophy was seen at 400 mg/kg, while mild focal atrophy also occurred at 100 and 200 mg/kg.[30] The major VPA-induced pathologic lesions were bilateral testicular atrophy, with reduced or absent spermatogenesis, and secondary atrophy of the prostate and epididymis. As only minor changes were observed in other organs, it was concluded that the testes should be considered a target organ for VPA toxicity. These results are consistent with those of other studies, which have shown reduced spermatogenesis and testicular atrophy of dogs at VPA doses greater than 90 mg/kg.[31] The findings from Walker et al.[30] are supported by some results from the study of Nishimura et al.,[20] which demonstrated reduced testicular weight and reduced weights of epididymis, seminal vesicles, and prostate after long-term VPA treatment of rats.

The effects of long-term VPA treatment on testicular morphology have been studied in detail in rats.[32] Male Wistar rats were fed perorally twice daily for 90 days with either VPA (200 or 400 mg/kg), or lamotrigine (LTG) (5 mg/kg). The main finding

was a marked, highly significant, testicular atrophy, with a 51% reduction in testicular weight, in the VPA high-dose treatment group of animals, with no changes in the other treatment groups. Widespread testicular atrophy, with histologically verified spermatogenetic arrest, was recorded in the VP high-dose treatment group, but changes were not observed in the interstitium, including the Leydig cells. Furthermore, no significant changes in the testis were seen either in the low-dose VPA-treatment group, or in the LTG-treatment group. No changes were detected in the liver, heart, lungs, lymphatic nodes, pancreas, kidney, or brain, which supports the observations of Walker et al.[30] of the testis being a target organ for VPA toxicity. The difference in effect between VPA and LTG also indicates a drug-specific effect of VPA, independent of epileptic activity, since all the animals used were healthy, non-epileptic rats.

Effects of AEDs on ovarian morphology

A drug-specific effect of AEDs on ovarian morphology has been studied in detail only for VPA, instigated by the ongoing discussions regarding development of polycystic ovaries following VPA treatment in female epilepsy patients.

In the study of Snyder and Badura,[25] chronic VPA administration in female rats for 8 weeks led to a significant reduction in uterine weight after 4 and 6, but not 8 weeks of age. In the ovary, VPA was associated with a significant reduction in both follicles and corpora lutea after 6 weeks of age. Again, no significant changes were observed after 8 weeks of age. In the same study, humerus length was also measured, and demonstrated reduced growth after 4 and 6, but not 8, weeks of age. Considering these results together, the effects of VPA on gonadal and skeletal growth indicated that pubertal maturation had been slowed, but not completely halted.

Long-term VPA treatment of adult rats has been shown to induce an increase in the number of cystic formations in the ovary. In the study of Taubøll et al.,[33] rats were fed perorally with either control solution, or VPA (50 or 200 mg/kg) once daily for 90 days,[34] resulting in mean serum VPA concentrations of 72 μmol/l in low-dose treated animals and 200 μmol/l in high-dose treated animals 4 h after the last dose. In the high-dose group, additional serum VPA concentration measurements 2, 4, 6, and 24 h after the last dose were 999, 200, 157, and 3 μmol/l, respectively. The main finding of this study was a significant, dose-dependent increase in the number of cystic formations in the ovaries.

In a second study, female Wistar rats were fed perorally with either 200 or 300 mg/kg VPA, or 5 mg/kg LTG, or control solution twice daily, for 90 days.[34] After a dose of 200 mg/kg VPA, mean serum concentrations of VPA were 1444, 819, and 241 μmol/l after 2, 4, and 6 h. Corresponding values after 300 mg/kg were 1742, 967, and 330 μmol/l. After 12 h, serum concentrations had reduced to 3 μmol/l. Mean serum concentration of LTG 6 h after last dose was 38.6 μmol/l. Again, a significant, dose-dependent increase in the number of follicular cysts in the ovaries was observed after VPA treatment. This finding was strengthened by a concomitant reduction in the number of corpora lutea. No effects were observed after LTG treatment. A detailed analysis of a series of other tissues, including liver, kidney, pancreas, lymphatic tissue, heart, brain, and cerebellum did not reveal any changes. The study therefore showed that the effect of VPA was drug-specific, independent of epileptic activity, and that the ovaries were a possible target organ.[34] This is further supported by the observations from another study[23] which demonstrated that VPA treatment of rats, at a total dose of 300 mg/(kg day) for 30 days, resulted in an increase in the number of cystic follicles in the ovary.

However, in contrast, in another study in which a different animal model, the Rhesus monkey, was used, 12—15 months of VPA treatment did not induce any discernible changes in ovarian morphology.[24]

Endocrine effects and possible mechanisms of action of AEDs on sex steroid hormones

Several AEDs have been shown to exert a direct effect on sex steroid hormone production. The first study in animals to address this question was published in 1990, and investigated the effects of VPA, CBZ, and PHT on different steps of testosterone biosynthesis in isolated rat Leydig cells.[35] Using sub-maximally stimulating concentrations of human chorionic gonadotropin (hCG), resulting in physiological testosterone secretion rates, half-maximal inhibition of testosterone formation occurred in the presence of 15 μM CBZ, 180 μM PHT, or 900 μM VPA. Only the values for CBZ were in the clinically therapeutic range. CBZ acted primarily at a target between cyclic AMP formation and cholesterol conversion to androgens, while PHT acted by competitive interaction at the cytochrome P450XVIII, which converts progesterone to androgens.

Some, but not all, benzodiazepines alter androgen production. This may be due to selective effects of the different benzodiazepines on different types of benzodiazepine receptors. Peripheral-type benzodiazepine receptors have been characterized in various tissues, including the ovary and testis. CZP,

that acts only on the central-type benzodiazepine receptors, failed to affect androgen production, whereas diazepam, which binds to both central and peripheral benzodiazepine receptors, was able to induce a significant increment of basal and hCG-stimulated testosterone production.[36] However, other studies have found that chronic treatment of male rats with diazepam, administered intraperitoneally, is associated with lowered serum testosterone levels.[37] As there was no difference in serum LH and FSH, or in the hypothalamic luteinising-hormone releasing hormone (LHRH) content, it was suggested that diazepam could act directly on the testicular interstitial cells to reduce testosterone production.[37] This theory is supported by data demonstrating that the peripheral-type benzodiazepine receptor agonist Ro 5-4864 affects androgen production from suspensions of isolated rat interstitial cells, suggesting that benzodiazepines acting on peripheral benzodiazepine receptors have a direct effect on Leydig cells.[36]

Long-term VPA treatment in female rats has been shown to increase the testosterone to estrogen ratio markedly, mainly by decreasing estrogen levels.[38] With respect to the gonadotropins, there was no increase in LH after VPA treatment, but there was a trend towards reduced LH levels at high VPA doses, which reached statistical significance at the 400 mg/(kg day) dose. No change was seen in FSH levels. LTG, however, did not affect the hormones studied. Taken together, these results following long-term VPA treatment, of a pronounced reduction in estrogen, a marked increase in the testosterone to estrogen ratio, and only minor effects, if any, on gonadotrophins, might suggest a direct effect of VPA on peripheral sex steroid hormone production in the ovary.

In order to investigate the possibility of a direct effect of VPA on follicular steroidogenesis in more detail, the secretion of testosterone and estradiol from isolated porcine ovarian follicles was studied.[39] Using concentrations from approximately 600–1500 μmol/l VPA, it was shown that VPA increased testosterone and reduced estradiol secretion, and reduced the conversion of testosterone to estradiol. These observations indicate a direct effect of valproate VPA on steroidogenesis in the ovary.

A direct effect of VPA has been further supported by the findings of Hattori et al.,[40] with respect to the presence of the enzyme microsomal epoxide hydrolase (mEH) in human ovaries. mEH is important in detoxification of several substances, and several studies have shown that VPA inhibits mEH activity.[41] Hattori et al.[40] demonstrated that human granulosa cells expressed mEH, and that the inhibition of mEH suppressed conversion of testosterone to estradiol. With VPA as a mEH inhibitor, this may be a pathway by which VPA reduces estrogen levels, and thereby increases the testosterone to estrogen ratio. This will lead to an androgen-dominant microenvironment in the ovary, and thereby possibly result in polycystic changes occurring without an increase in LH levels.[40]

Again, however, the study by Ferin et al. using Rhesus monkeys[24] suggests an absence of endocrine effects associated with long-term treatment with VPA. However, only seven animals were included in this study, and there was a trend toward increased testosterone to estrogen ratio, lower estrogen levels, and increased LH/FSH ratio. In addition, body weight was significantly increased.

The possible direct effects of other AEDs, especially the "newer" ones, should also be studied. Levetiracetam (LEV) is of particular interest as it binds to the synaptic vesicle protein SV2A.[42] SV2A is widely distributed in the nervous system and also in endocrine tissue. If LEV binds to SV2A vesicle protein in the ovary and/or in the pituitary gland or hypothalamus, this may provide a link between the drug and endocrine function at either a central or peripheral level. In a recent paper, we showed for the first time that LEV may affect basal, but not gonadotropin-stimulated, testosterone and estrogen secretion from porcine ovarian follicular cells.[43] The clinical consequence of this may be that this AED could be an alternative therapy for women of fertile age since they will have gonadotropins present, and that further studies of LEV are necessary, especially in young girls with low gonadotropin levels. It must be emphasised that our finding should be confirmed in further studies before any definite conclusions on the clinical relevance of this observation can be drawn.

Recently, two papers have indicated possible endocrine effects of topiramate (TPM). In female rats, treatment with TPM (100 mg/kg) for 12 weeks reduced fertility and ovarian weight.[44] In male rats, the same dose given for 8 weeks reduced spermatogenesis, sperm motility, and the weight of reproductive organs.[45] The serum concentrations of TPM were not measured, and the clinical relevance of the observations is uncertain. However, these studies do underline the importance of investigating all AEDs more closely with regard to possible endocrine effects.

AEDs may, of course, also influence sex steroid hormones via a centrally mediated effect, by altering gonadotropin secretion. A male rat study[38] demonstrated a dose-dependent increase in LH after long-term VPA treatment and also an increase in FSH at the highest dose. In isolation, this finding

might imply a direct effect of the drug on the central nervous system. However, the increased gonadotropin levels might also be considered as a compensatory mechanism, related to the marked peripherally induced effects seen in the animals with testicular atrophy and spermatogenetic arrest, and unaltered testosterone levels despite a threefold increase in LH. Lack of a centrally mediated effect is further supported by the finding of unchanged FSH and prolactin levels in mice after 8 weeks of VPA treatment at doses that led to reduced pubertal maturation and alterations in ovarian and testicular function.[25] In contrast, long-term VPA treatment in rats has been shown to delay gonadotropin-releasing hormone (GnRH) cell morphological maturation within the hypothalamus in young, but not adult, mice.[46] Long-term, low-dose, VPA or CBZ treatment in rats has also been shown to increase prolactin levels, and to reduce FSH and LH levels, in male rats.[18] A centrally mediated effect of AEDs on sex hormone regulation can therefore not be excluded and should be explored in further studies.

Concluding remarks—animal studies

Several animal studies have shown that both the epilepsy itself, and many AEDs, affect reproductive endocrine function in both males and females. Although care should always be taken when applying data from animal experiments to the human situation, animal models provide a unique possibility to investigate the independent effects of the epilepsy itself, and the effects of AEDs in isolation, without confounding factors. By constantly comparing results from clinical and animal studies, and by developing appropriate animal models, several mechanistic questions regarding the complex interplay between epilepsy, hormones, and AEDs can be explored.

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